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Abstract

The bioaccumulation, tissue and subcellular distributions of Ag were investigated in the king scallop *Pecten maximus* from the Bay of Seine fishery area (France) in laboratory and in field conditions. Experimental investigations with the radiotracer ^{110m}Ag showed that the scallop readily concentrated Ag when exposed via seawater and to a much lower extent when exposed via sediment. Retention of the metal incorporated via all tested contamination pathways was shown to be very strong, but the assimilation efficiency of Ag ingested with food was found to be tightly depending on the phytoplankton strain used to feed the scallops (74 and 33% with *Skeletonema costatum* and *Isochrysis galbana*, respectively). The uptake and depuration kinetic parameters determined in the laboratory experiments were used to run a global bioaccumulation model. The latter indicated that the major uptake pathway of Ag in *P. maximus* was strongly depending on the food quality. Indeed, when fed the diatom *S. costatum* which was characterised by a high affinity for Ag (high distribution constant K_{df}) the relative contribution of the feeding pathway reached 98% of the global Ag bioaccumulation. In contrast, when fed *I. galbana* which displayed a lower K_{df} than *S. costatum*, dietary Ag was retained to a lesser extent by *P. maximus*, and seawater appeared as the major contributing uptake pathway. In wild scallops collected from reference and contaminated sites, Ag was mainly concentrated in the digestive gland and secondarily in the gills, and was mainly found associated with the insoluble subcellular fraction in all the scallop tissues.

Keywords: Metal; bioaccumulation; exposure pathway; relative contribution; bivalves; Pectinid.

1. Introduction

Inputs of Ag in the marine environment through sewage sludge from the coastal cities is of environmental concern (Sañudo-Wilhelmy and Flegal, 1992; Luoma et al., 1995), especially as this metal is well known to be one of the most toxic elements (e.g., Warnau et al., 1996b; Ratte, 1999). Once in the marine environment, Ag tends to accumulate in the sediments and to concentrate in benthic deposit- and filter-feeders (Bryan, 1985; Luoma et al., 1995). In bivalves, Ag concentrations were shown to be closely related to the contamination degree of their environment (e.g., Young and Jan, 1979; Bustamante and Miramand, 2005) and the occurrence of Ag in these organisms may therefore be used as a valuable proxy of urban contamination (Sañudo-Wilhelmy and Flegal, 1992; Luoma et al., 1995).

Among filter-feeders, the Pectinidae are known to accumulate very high concentrations of Ag (e.g., Brooks and Rumsby, 1965; Bryan, 1973; Bustamante and Miramand, 2005; Metian et al., 2008). This accumulation is especially strong in the digestive gland, in which Ag concentrations are generally above 60 µg g^{-1} dry wt, without apparent toxic effects (Brooks and Rumsby, 1965; Bryan, 1973; Bustamante and Miramand, 2005). It is well documented in bivalves that Ag is detoxified via binding with thiol groups and is then precipitated in the connective tissues, particularly in the digestive system (e.g., Berthet et al., 1992). Ag is also known to display high affinity for metallothioneins (MTs). As these proteins have been reported to occur in high concentrations in Pectinidae (e.g., Viarengo et al., 1993; Ponzano et al., 2001), MTs could also contribute significantly to Ag detoxification in this group.

Overall, although Ag bioaccumulation has been investigated in several bivalves (e.g., Wang et al., 1996; Reinfelder et al., 1997), little information is available on Ag bioaccumulation and detoxification processes in Pectinidae. In particular, uptake and depuration kinetics of Ag are only documented for one species (the variegated scallop *Chlamys varia*) exposed via seawater (Metayer et al., 1990). In the latter study, the Ag exposure concentrations were very high (1 to 1,000 $\mu g\ l^{-1}$) and far higher than concentrations actually found in marine waters, even in heavily contaminated areas (Ratte, 1999; Gorsuch et al., 2003). Furthermore, such elevated Ag concentrations resulted in toxic effects in marine bivalves (e.g., Eisler, 1996). Nevertheless, *C. varia* displayed a strong retention capacity for Ag in its tissues (Bustamante and Miramand 2005), where it was stored as Ag sulphide (Martoja et al., 1989).

The present work investigated the processes governing Ag bioaccumulation in the king scallop *Pecten maximus*, through both field and laboratory approaches. As a first step, Ag bioaccumulation was assessed in controlled laboratory conditions during experimental exposures via seawater, food or sediment, using the ^{110m}Ag γ-emitting radiotracer. This approach allowed determining the uptake and depuration kinetic parameters of Ag as well as its tissue and subcellular distributions for each contamination pathway. The parameters were then used to feed a global bioaccumulation model in order to delineate the relative contribution of the three different pathways of exposure. In a complementary field study, wild scallops were collected from a Ag-clean site and a Ag-contaminated site and analysed for their concentrations, and tissue and subcellular distributions in Ag and MTs. Results from both field and laboratory studies were then combined in order to better understand the bioaccumulation of Ag in *P. maximus* as well as to further assess whether *P. maximus* could be used as a reliable bioindicator species of marine Ag contamination.

2. Materials and methods

2.1. Laboratory experiments

King scallops (*Pecten maximus*) were collected on the Atlantic coast of France (Bay of Seine) and acclimated for 3 weeks to laboratory conditions. During this period, scallops were fed daily a microalgal diet ($5 \cdot 10^4$ cells ml^{-1}) composed of the Bacillariophyceae *Skeletonema costatum* and the Prymnesiophyceae *Isochrysis galbana*.

Thirteen individuals (mean weight: 208 ± 46 g) were placed in a 50-l aquarium and exposed for 7 d to 0.4 kBq l^{-1} ^{110m}Ag dissolved in filtered (0.45 µm) seawater, according to the methodology described in Warnau et al. (1996c, 1999). At each daily renewal of seawater and spike, the scallops were fed briefly (30 min) *S. costatum* and *I. galbana* ($5 \cdot 10^4$ cells ml^{-1}) in clean seawater. At the end of this period, 4 scallops were dissected and the remaining scallops were placed in non contaminated, flowing seawater to follow Ag depuration for 36 d. During the depuration period, scallops were fed daily *S. costatum* and *I. galbana* ($5 \cdot 10^4$ cells ml^{-1}).

Fourteen scallops (mean weight: 118 ± 5 g) were placed in a 50-l aquarium and exposed for 13 d under flowing seawater to sediment previously spiked with ^{110m}Ag (31 Bq ^{110m}Ag g^{-1} wet wt) according to the method described in Danis et al. (2005) and adapted as in Metian et al. (2007). After 13 d, 4 individuals were dissected, and the remaining individuals were placed in depuration conditions for 49 d (clean sediment under flowing seawater, daily feeding on *S. costatum* and *I. galbana* at $5 \cdot 10^4$ cells ml^{-1}). The spiked sediment was mainly composed (95.8%) of grain which size ranged from 76 to 302 µm; its dry/wet wt ratio was 0.80. At the end of the 49-d depuration period, 4 scallops were dissected.

Eighteen scallops (mean weight: 170 ± 69 g) were placed in a 50-l aquarium and exposed to radiolabelled food (phytoplankton; $5 \cdot 10^4$ cells ml^{-1}) of contrasting nature in order to encompass the complexity of the phytoplankton diet of scallops (Mikulich and Tsikhon-Lukamina, 1981; Shumway et al., 1987). In this respect, one culture of *S. costatum* (Si-walled diatom) and one of *I. galbana* (naked flagellate) were previously exposed during their exponential growth phase to 5 and 4 kBq ^{110m}Ag l^{-1} for 10 and 7 d, respectively, and then separated from the spiked seawater (1-µm filtration) according to the method described in

Metian et al. (2007). Nine scallops were fed *S. costatum* and 9 were fed *I. galbana*. After a 2-h feeding on one or the other phytoplankton strain (pulse-chase feeding method, Warnau et al., 1996c; Metian et al., 2007), all scallops were whole-body γ-counted (see below, end of this section) and then placed in non contaminated, flowing seawater conditions, with daily feeding on *S. costatum* and *I. galbana* ($5 \cdot 10^4$ cells ml^{-1}). Four scallops were dissected after 21 d (*S. costatum*) or 16 d (*I. galbana*).

Preliminary feeding experiments were also carried out in order to assess the ingestion rate (IR) of both phytoplankton strains by *P. maximus*. In this respect, four batches of three scallops were each placed in a 20-l aquarium (constantly aerated) supplied with $5 \cdot 10^4$ cells ml^{-1}. In each aquarium, three samples of seawater (5 ml) were collected at t_0 and after 30 and 60 min. The cell density was counted in each sample under a light microscope, using a Thoma cell, and the difference between final and initial cell densities was converted to IR ($g\ g^{-1}\ d^{-1}$).

During the three experiments (seawater, food and sediment), samples of water and/or sediment were collected twice a day during both exposure and depuration phases and radioanalysed in order to check for any possible variation in ^{110m}Ag activity in the microcosms (exposure phases) or for any possible ^{110m}Ag recycling via seawater (exposure phase via sediment and depuration phase for the three pathways). Data obtained during the exposure periods were used to determine the time-integrated activity (Rodriguez y Baena et al., 2006) and to calculate the concentration (seawater exposure) and transfer (sediment exposure) factors (see Metian et al., 2007).

In the three experiments (seawater, sediment and food), the dissected scallops were used to determine the distribution of ^{110m}Ag among the different body compartments (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues). In all experiments, the distribution of ^{110m}Ag between the soluble and insoluble subcellular fractions was also determined in the digestive gland, according to the method described by Bustamante and Miramand (2005). Briefly, a part of the gland was homogenized individually with a mortar and pestle on ice with 10 ml of 0.02M Tris–HCl buffer at pH 8.6. The homogenates were then centrifuged at 30,000 g for 1 h at 4°C in a Sorvall RC28S ultracentrifuge to separate particle-free supernatant (cytosol; i.e., the soluble fraction) from the pellet (i.e., the insoluble fraction). Aliquots of the homogenates, cytosols, and pellets were radioanalysed using a γ spectrometer (see here below).

Biokinetic experiments were carried out using a high-specific activity ^{110m}Ag radiotracer purchased from Isotope Product Lab, Germany (^{110m}Ag as $AgNO_3$, $T_{½}$ = 249.8 d).

Radioactivity in live scallops that were radioanalysed daily during uptake and depuration experiments and in dissected and centrifuged samples were counted using a high-resolution γ-spectrometry system consisting of four coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6).

The radioactivity was determined by comparison with standards of known activity and of appropriate geometry. Measurements were corrected for counting efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error less than 5% (Warnau et al., 1999).

2.2. Field study

Ten *P. maximus* were collected by SCUBA diving in 2 different locations the Bay of Seine in July 2004: a site near the estuary (49°35’0” N ; 0°10’0” W) which is known to be contaminated by chemicals, and a reference site (49°33’0” N ; 1°0’0” W) located far from the Seine river mouth in the western part of the Bay (Gilliers et al., 2006).

Scallops were frozen on board (-20°C) and dissected immediately upon return to the laboratory in order to separate the digestive gland, gills, kidneys, gonad and adductor muscle;

the other tissues and organs constituted the remaining soft tissues. All dissected tissue samples were freeze-dried and grounded to powder. Two aliquots (ca. 150 mg dry wt) of each tissue were treated as described above to separate the soluble and insoluble subcellular fractions according to the method of Bustamante and Miramand (2005).

Metallothionein-like protein (MT) concentrations were determined in heat-treated supernatants by differential pulse polarography, which allows quantifying the cysteinic residues (Thompson and Cosson, 1984; Temara et al., 1997). Polarographic measurements were carried out with a PARC Model 174A analyzer and a PARC EG&G Model 303 static mercury drop electrode (SMDE). Standard quantification of MTs was based on rabbit liver metallothionein (Sigma, M-7641) by the standard addition method. MT concentrations in scallop tissues were expressed as µg g^{-1} dry wt.

In addition, Ag was analysed in supernatants and pellets after acidic digestion with 4:1 (v:v) ultrapure 65% HNO_3 and 70% $HClO_4$ at 80°C for 2 d. The acids were then evaporated until dryness and the residues were dissolved in 0.3 N ultrapure 65% HNO_3. Metal analyses were carried out using an atomic absorption spectrophotometer Hitachi Z-5000 with Zeeman background correction. Reference tissues, dogfish liver (DOLT-3, NRCC) and lobster hepatopancreas (TORT-2, NRCC) were treated and analysed in the same way as the samples. Results were in good agreement with the Ag certified values: recoveries ranged from 93 to 106% (n = 10). Detection limit was 0.005 µg Ag g^{-1} dry wt. Ag concentrations in scallop tissues were expressed as µg g^{-1} dry wt.

2.3. Data analysis

Uptake and depuration kinetics of the different exposure experiments were fitted using the kinetic models and statistical methods as described by Warnau et al. (1996a,c) and Metian et al. (2007).

The relative contribution of each uptake pathway was determined using the global bioaccumulation model originally proposed by Thomann (1981) and Landrum et al. (1992), and further used and developed by other authors (e.g., Thomann et al., 1995; Wang et al., 1996; Reinfelder et al., 1998; Metian et al., JEMBE or MEPS), in which three different uptake pathways (seawater, food and sediment) were considered. In this model, the introduction of the term A_{0l} allowed considering only the fraction of the metal that was actually incorporated by the organisms exposed via seawater, sediment and food. The metal concentrations in sediment and food were calculated using their distribution coefficients (K_d and K_{df}, respectively). The concentration in sediment, C_s (Bq g^{-1} wet wt) was determined according to the relation:

Supprimé : developed by Landrum et al. (1992) and reviewed by Thomann et al. (1995) and Wang et al. (1996)

$$C_s = C_w K_d \qquad \text{(eq.1)}$$

where C_w is the concentration of the radiotracer in experimental spiked seawater (Bq l^{-1}) and K_d the Ag distribution coefficient in sediment according to IAEA (2004).

The concentration in the phytoplankton strains, C_f (Bq g^{-1} wet wt) was determined according to the relation:

$$C_f = C_w K_{df} \qquad \text{(eq.2)}$$

where K_{df} is the Ag distribution coefficient in the phytoplankton strain (*S. costatum* or *I. galbana*) that was measured in our experiments.

Total Ag concentration in the organisms (i.e. the sum of each concentration resulting from the uptake through the different pathways) was defined as:

$$C_t = C_{w,ss} + C_{s,ss} + C_{f,ss} \qquad \text{(eq.3)}$$

where $C_{w,ss}$, $C_{s,ss}$ and $C_{f,ss}$ are the steady-state metal concentrations (Bq g^{-1}) in scallops resulting from uptake via seawater, sediment and food, respectively. The latter were computed according to the relations:

$$C_{w,ss} = (A_{0l,w}\ k_{u,w}\ C_w) / k_{e,w} \qquad \text{(eq.4)}$$

$$C_{s,ss} = (A_{0l,s}\ k_{u,s}\ C_s) / k_{e,s} \qquad \text{(eq.5)}$$

$$C_{f,ss} = (AE\ IR\ C_f) / k_{e,f} \qquad \text{(eq.6)}$$

where A_{0l} is the metal fraction (%) strongly retained in scallop tissues, k_u is the uptake arte constant (d^{-1}), k_e is the depuration rate constant (d^{-1}), AE is the metal assimilation efficiency from food (i.e., $A_{0l,f}$; %), and IR is the ingestion rate (g $g^{-1}d^{-1}$), and where w, s, and f subscripts refer to seawater, sediment and food as exposure source, respectively.

The relative contributions of the three pathways (dissolved, sediment and food) were then assessed according to the following relations:

$$\%_{\text{seawater}} = C_{w,ss} / (C_{f,ss} + C_{s,ss} + C_{w,ss}) \qquad \text{(eq.7)}$$

$$\%_{\text{sediment}} = C_{s,ss} / (C_{f,ss} + C_{s,ss} + C_{w,ss}) \qquad \text{(eq.8)}$$

$$\%_{\text{food}} = C_{f,ss} / (C_{f,ss} + C_{s,ss} + C_{w,ss}). \qquad \text{(eq.9)}$$

In the field study, one-way ANOVA was used to test for differences among Ag and MT concentration data using the corresponding routines in the software Statistica® 6.1. Level of significance for the statistical analyses was always set at $\alpha = 0.05\%$.

3. Results

3.1. Laboratory experiments

3.1.1 Exposure via seawater

The whole-body uptake kinetics of ^{110m}Ag in *P. maximus* were best fitted by a first-order saturation exponential equation ($R^2 = 0.71$). The kinetics and the estimates of its parameters and associated statistics are shown in Figure 1a. Although the steady-state was not reached during the time course of the experiment, the concentration factor at steady-state could be estimated with reasonable precision ($CF_{ss} = 801 \pm 164$). After 7 d of exposure, the measured CF_{7d} was 620 ± 210 in the whole-body individuals, 3,740 ± 1,020 in the whole soft parts, 56,600 ± 13,400 in the digestive gland and 4,440 ± 3,110 in the gills (data not shown). At the end of the uptake period, ^{110m}Ag was mainly located in the soft tissues (98 ± 2% of the total ^{110m}Ag content), among which the digestive gland contained the major part of the tracer (71 ± 7%), followed by the gills (13 ± 7%) (Fig. 2).

During the 36-d depuration period following seawater exposure, the whole-body depuration kinetics of ^{110m}Ag was best described by a bi-exponential equation ($R^2 = 0.22$; Fig. 1a). The results showed that the major part of ^{110m}Ag was efficiently incorporated ($A_{0l} = 82\%$) and strongly retained in the tissues of *P. maximus* (biological half-life not significantly different from infinite). At the end of the depuration period, the body distribution of ^{110m}Ag indicated that the digestive gland stored the main part of the total body ^{110m}Ag (80 ± 8%), as previously observed at the end of the uptake phase (71 ± 7%) (Fig. 2).

3.1.2. Exposure via the sediment

The whole-body uptake kinetics of ^{110m}Ag bound to sediments were best fitted by a first order saturation exponential equation (R^2 = 0.81) that reached a steady-state equilibrium after ca. 10 d of exposure (Figure 1b). TF_{13d} *in toto* and in the whole soft parts were 0.22 ± 0.07 and 0.69 ± 0.19, respectively. Among the different organs and tissues, the highest TF_{13d} was found for the digestive gland (11.8 ± 4.9), which contained 65 ± 13% of the whole-body ^{110m}Ag activity (Fig. 2).

The whole-body depuration kinetics after exposure via spiked sediment were best described by a mono-exponential equation (R^2 = 0.64; Fig. 2). The results indicated that 90% of ^{110m}Ag previously bioaccumulated were efficiently retained, with a biological half-life of 40 d. At the end of the 31-d depuration period, the body distribution of ^{110m}Ag clearly showed that the major part of the metal (ca. 66 ± 8%) was distributed in the digestive gland, the latter proportion being not significantly different to that observed at the end of the exposure period (Fig. 2).

3.1.3. Exposure via the food

Prior to feeding the scallops with one out of the two selected diets (*Skeletonema costatum* or *Isochrysis galbana* strain), the phytoplankton cultures were exposed to ^{110m}Ag for 7 and 10 d, respectively. At the end of the exposure period, the distribution coefficient between phytoplankton and water (K_{df}) was determined in order to assess the efficiency of their affinity for Ag. K_{df} was 6.86 10^5 for *S. costatum* and 4.43 10^4 for *I. galbana*.

In addition, the preliminary assessment of the ingestion rate (IR) of phytoplankton indicated that *P. maximus* ingested both *S. costatum* and *I. galbana* with similar ($p = 0.01$) rates, i.e. IR = 0.0404 g g^{-1} d^{-1}.

Following the pulse-chase feeding using either *S. costatum* or *I. galbana* as food, the whole-body depuration kinetics of ^{110m}Ag were best fitted using a bi-exponential equation (R^2 = 0.46 for *S. costatum* and 0.93 for *I. galbana*) (Fig. 1c). The assimilation efficiency (AE) of ^{110m}Ag ingested with food was higher when scallops were fed *S. costatum* than *I. galbana* (i.e. AE = 72 vs. 33%). Furthermore, when ingested with *S. costatum*, the tracer was much strongly retained in *P. maximus* tissues ($T_{b½l}$ not different from infinite; Fig. 1c1) than when ingested with *I. galbana* ($T_{b½l}$ = 11 d; Fig. 1c2). ^{110m}Ag was mainly distributed in the digestive gland (> 93%) with both food sources (Fig. 2).

3.1.4. Subcellular distribution

For all three exposure modes, ^{110m}Ag was always mainly associated with the insoluble subcellular fraction of the digestive gland cells (84 to 98%; see Fig. 2).

3.1.5. Global bioaccumulation model

In order to assess the relative contribution of each uptake pathway to the global Ag accumulation in *P. maximus*, the different kinetic parameters obtained for the three exposure modes to Ag (seawater, food, and sediment) were used to feed a global bioaccumulation model (see section 2.3.) along with other parameters such as the distribution coefficient of Ag in sediment (K_d = 2 10^4; IAEA, 2004), the Ag K_{df} in phytoplankton strains (K_{df} = 6.86 10^5 for *S. costatum* and 4.43 10^4 for *I. galbana*; present study) and the ingestion rate of phytoplankton in scallops (IR = 0.0404 g $g^{-1}d^{-1}$ for both strains; present study).

Results of the computations are shown in Figure 3. Most interestingly, the phytoplankton strain used as food source strongly influenced the modelling results. In fact, the latter food source was actually driving the outcomes of the modelling and the computations were thus performed considering one or the other phytoplankton strain. With *S. costatum*, the feeding pathway was by far the major contributor (98%) to the global bioaccumulation of Ag in *P. maximus* (Fig. 3a), whereas seawater was the main contributor (63%) followed by sediment (28%) when the scallops were fed *I. galbana* (Fig. 3b).

3.2. Field study

Concentrations of Ag in the tissues and organs of *P. maximus* collected from the two sampling sites are given in Table 1. Scallops from the contaminated zone showed significantly higher ($p = 0.004$) Ag concentrations in their whole soft tissues than that from the reference site. Such a difference was also significant for the digestive gland ($p = 0.004$), the gills ($p = 0.0001$) and the remaining tissues ($p = 0.004$). In both sites, the highest Ag concentrations and loads were found in the digestive gland and the lowest in the gonad and the adductor muscle (Table 1).

In all the tissues and organs analysed, Ag was mainly associated with the insoluble subcellular fraction, which contained from 77% (adductor muscle) up to 97% (digestive gland) of the metal (Table 1). Interestingly, the subcellular distribution of Ag was not significantly different between the two sites for all organs and tissues but the gills ($p = 0.01$) (Table 1).

The concentrations in metallothionein-like proteins varied from 750 µg g^{-1} dry wt in the adductor muscle up to 7,450 µg g^{-1} dry wt in the digestive gland (Table 1). The concentrations were only significantly different between the two sites for the gills ($p = 0.007$), gonad (p =

0.041) and remaining tissues ($p = 0.013$), with the contaminated site showing the highest values.

4. Discussion

Important Ag releases in the Bay of Seine occur through the Seine River which is one of the most heavily contaminated rivers in Europe (Roux et al., 2001). The average Ag concentrations measured in this study in the whole soft tissues of *Pecten maximus* from the Bay of Seine ranged from 2.6 to 7.2 µg g^{-1} dry wt). The digestive gland, gills and remaining tissues of the scallops collected in the vicinity of the estuary (i.e. the contaminated site) displayed Ag concentrations that were two to ten times higher than those from the reference site (Table 1). This spatial variation of Ag concentrations in scallop tissues has already been reported in other species such as *Chlamys varia*, which was therefore considered as a good biomonitor species for this metal (Bustamante and Miramand, 2005) and thus for urban contamination. Indeed, Ag is generally considered as a reliable proxy for domestic inputs in coastal waters due to Ag-enrichment in the sewage sludge from coastal cities (e.g., Sañudo-Wilhelmy and Flegal, 1992; Luoma et al., 1995; Andren and Bober, 2002).

Data on Ag concentrations have been reported for several scallop species such as *Hinnites giganteus* (Young and Jan, 1979), *C. varia* (Bustamante and Miramand, 2005), *Comptopallium radula* (Metian et al., 2008), and *P. maximus* (Segar et al., 1971; Bryan, 1973). The highest Ag concentrations were generally found in the digestive gland of these scallop species.

The digestive gland of the scallops collected at the reference and contaminated sites of the Bay of Seine displayed mean Ag concentrations of 32 and 89 µg g^{-1} dry wet, respectively. In the contaminated site, these digestive gland concentrations were rather elevated in comparison to data from the literature for *P. maximus*. Indeed, mean values previously reported were 8.9 and 13.6 µg g^{1} dry wt from the Irish Sea and from the English Channel, respectively (Segar et al., 1971; Bryan, 1973). However, in other scallop species, values ranged from 30 to 77 µg g^{1} dry wt (Bryan, 1973; Mauri et al., 1990; Bustamante and Miramand, 2004).

The gills of *P. maximus* from the contaminated site displayed very high Ag concentrations viz. similar to those measured in the digestive gland from the reference site. This strongly suggests that an important incorporation of the metal occurred through the dissolved pathway. Indeed, this organ is well-known to play a key role in dissolved metal accumulation in marine filter-feeders (Rainbow, 1990). This assumption is well supported by our laboratory experiments showing that *P. maximus* efficiently concentrated waterborne Ag (CF_{7d} > 3,700 in the whole soft parts). Uptake of dissolved Ag might occur in all the soft parts in direct contact with seawater, particularly at sites with high permeability such as the gills (Rainbow, 1990). However, in our experiments, most of the radiotracer (> 70%) was found in the digestive gland, which showed very high CF (> 56,000), even after a short exposure time of 7 d. This strongly suggests that translocation of the metal occurred from the tissues in direct contact with seawater (gills) towards the digestive gland, where the metal would have been detoxificated and stored. Interestingly, although uptake of Ag bound to sediment was weak (TF_{13d} in whole soft parts = 0.69 ± 0.19), most of the metal was found in the digestive gland after 13 d of exposure (65 ± 13%). TFs in scallop tissues were far lower (by approximately 3 orders of magnitude) than CFs calculated from seawater exposure, indicating that sediment-bound Ag is poorly bioavailable to *P. maximus*. This is consistent with results reported for other bivalve species, for example in oysters of the genus *Crassostrea* (Abbe and Sanders, 1990, Ettajani et al., 1992).

The major role of the digestive gland in Ag bioaccumulation and detoxification was especially evidenced by results of the trophic transfer experiments. When scallops were fed *S. costatum* and *I. galbana*, their digestive gland contained most of the metal remaining at the end of the depuration period (90 and 70%, respectively). However, both the assimilation efficiency (AE, %) and the retention capacity ($T_{b½}$, d) of the metal strongly depended on the phytoplankton strain used as food source, i.e. 72% and 268 d for *S. costatum* and 33% and 11 d, for *I. galbana*. Elevated AEs of Ag ingested with *S. costatum* could be due to the storage of the metal under bioavailable forms in the cytoplasm of the phytoplankton cells (Reinfelder and Fisher, 1991; Wang et al., 1996; Reinfelder et al. 1997). However, the gut residence time that appears to be longer for *S. costatum* than for *I. galbana* (see the much faster initial decrease in depuration kinetics after feeding on *I. galbana*; Fig. 1c) could also influence the metal assimilation efficiency (Wang and Fisher, 1996).

Seawater, sediment and food exposures all resulted in important Ag bioaccumulation in the digestive gland of *P. maximus*, in which the metal was always associated mainly with insoluble subcellular compounds whatever the exposure pathway, i.e. from 84 to 98% of the total Ag (see Fig. 2). In wild scallops from the Bay of Seine, Ag was similarly mainly associated with the insoluble subcellular fraction of the digestive gland cells of *P. maximus* collected in both the reference site and the contaminated one. No clear relationships were observed between Ag concentrations or subcellular distribution and MT concentrations (see Table 1). In particular, distribution of Ag in the subcellular soluble fraction (where MTs are located; Thompson and Cosson, 1984) was much higher (2 to 7 times) in adductor muscle than in digestive gland, although the muscular MT content was one order of magnitude lower than in digestive gland. This would suggest that, if any, the MTs are only slightly contributing to Ag sequestration and subcellular distribution in scallop tissues, which is in agreement with previously observations indicating that, in the digestive gland of bivalves, Ag was associated with lysosomes and partially combined with mineral or organic sulphurs within the connective tissues (Bryan, 1973; Ballan-Dufrançais et al., 1985; Berthet et al., 1992).

In the natural environment, the three contamination pathways studied here occur and contribute simultaneously to the global bioaccumulation of the metal in the organisms.

Through the use of the kinetic parameters that we obtained experimentally, along with published K_d for sediments (IAEA, 2004) and K_{df} and IR that we measured, the delineation of the relative contribution of the three exposure pathways has been assessed, using the model developed by Landrum (1992) that we adapted to take into account the three uptake routes. The results showed that food quality played a major role in determining the major route(s) of Ag accumulation in *P. maximus* (Figure 3). With a Si-walled diatom (*S. costatum*) as food source, the dietary pathway was the main pathway of Ag uptake (98%) whereas when scallops were fed a naked flagellate (*I. galbana*), the major contribution of the metal came from seawater (63%) and secondarily from sediment (28%). The difference in the outcomes of the two runs of the model are due to the higher K_{df}, AE and $T_{b½}$ characterising Ag ingested with *S. costatum*. The information available in the literature regarding diet composition of scallops in the wild is scarce. It mainly reports that the diet of wild scallops is complex and can display quite large variations both in space and time (e.g., Mikulich and Tsikhon-Lukamina, 1981; Shumway et al., 1987; Grant and Cranford, 1991). Hence, without proper characterisation of this diet, it still appears rather premature to use the modelling approach to explain the observations from the field. Nevertheless, the bioaccumulation model that we used here allowed considering the sediment pathway separately for the first time. Sediment was expected to be an important pathway of contamination for P. *maximus*, since: 1) it is living at the seawater-sediment interface and thus can accumulate sediment-bound metals either directly by contact with the mantle and tentacles when deployed (filtering posture) or indirectly after desorption and release to the seawater or porewater, 2) it filters and ingests metal-rich particles from both the water column and the sediment surface (Shumway et al., 1987) and 3) sediment generally displays metal concentrations that are several orders of magnitude higher than those reported in seawater (e.g., Luoma, 1989). However, the model clearly showed that sediment had a minor contribution in Ag bioaccumulation in *P. maximus*.

Acknowledgements

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Captions to Figures

Fig. 1. Uptake and depuration kinetics of ^{110m}Ag in *Pecten maximus*: (a) seawater pathway (mean concentration factor ± SD, n = 13 and mean % remaining activity ± SD, n = 9), (b) sediment pathway (mean transfer factor ± SD, n = 14 and mean % remaining activity ± SD, n = 10), and (c) food pathway (mean % remaining activity ± SD, n = 9) via two phytoplankton strains, (c1) *Skeletonema costatum* and (c2) *Isochrysis galbana*.

Uptake parameters. CF_{ss}: concentration factor at steady state; TF_{ss}: transfer factor at steady state; k_u: uptake rate constant (d^{-1}).

Depuration parameters (long-lived component). A_{0l}: activity (%) lost according to the long-lived component; k_{el}: depuration rate constant (d^{-1}); $T_{b\frac{1}{2}l}$: biological half-life (d), R^2: determination coefficient; *: parameters not significantly different ($p > 0.05$) from 0 (k_{el}) or from the infinite ($T_{b\frac{1}{2}l}$).

Fig. 2. ^{110m}Ag distribution (mean % ± SD; n = 4) among the different body compartments of *Pecten maximus* exposed via: (a) seawater (end of uptake and depuration periods), (b) sediment (end of uptake and depuration periods) and (c) the food (end of the depuration after feeding on *Skeletonema costatum* -SKE- and *Isochrysis galbana* -ISO).

Fig. 3. Relative contribution of the different exposure pathways to global Ag bioaccumulation in *Pecten maximus* when (a) *Isochrysis galbana* or (b) *Skeletonema costatum* are considered as food source.

Table 1. *Pecten maximus*. Concentration (mean ± SD; µg g^{-1} dry wt; n = 10), tissue distribution (mean ± SD; %; n = 10), proportion of Ag associated to insoluble compounds (mean ± SD;% ; n = 10) and metallothionein-like protein (MT) concentrations (mean ± SD; µg g^{-1} dry wt; n = 10) in scallops from a contaminated and a reference sites in the Bay of Seine.

	Ag concentration (µg g^{-1} dry wt)		Proportion of Ag body burden (%)		Proportion of insoluble Ag (%)		MT concentration (µg g^{-1} dry wt)	
	Contaminated	Reference	Contaminated	Reference	Contaminated	Reference	Contaminated	Reference
Digestive gland	89 ± 29	32 ± 11	78 ± 11	86 ± 5	94 ± 3	97 ± 2 ^{NS}	5,450 ± 1,200	7,450 ± 1,200 ^{NS}
Gills	31 ± 14	3.1 ± 0.7	18 ± 10	6 ± 2	96 ± 3	92 ± 2	1,500 ± 180	1,100 ± 170
Kidneys	8.5 ± 3.2	5.7 ± 2.6 ^{NS}	1 ± 0	2 ± 1	86 ± 4	78 ± 10 ^{NS}	2,580 ± 440	2,420 ± 670 ^{NS}
Gonad	3.5 ± 1.1	2.4 ± 1.0 ^{NS}	1 ± 0	1 ± 0	87 ± 3	88 ± 3 ^{NS}	3,070 ± 310	2,150 ± 760
Adductor muscle	0.6 ± 0.4	0.4 ± 0.1 ^{NS}	< 1	1 ± 0	89 ± 5	77 ± 13 ^{NS}	830 ± 40	750 ± 150 ^{NS}
Remaining tissues	1.4 ± 0.3	0.7 ± 0.3	3 ± 1	4 ± 3	92 ± 2	89 ± 4 ^{NS}	2,000 ± 110	1,620 ± 240
Whole soft parts	7.2 ± 1.8	2.6 ± 0.7	100	100	-	-	-	-

NS: non significant difference between contaminated and reference sites ($p > 0.05$)

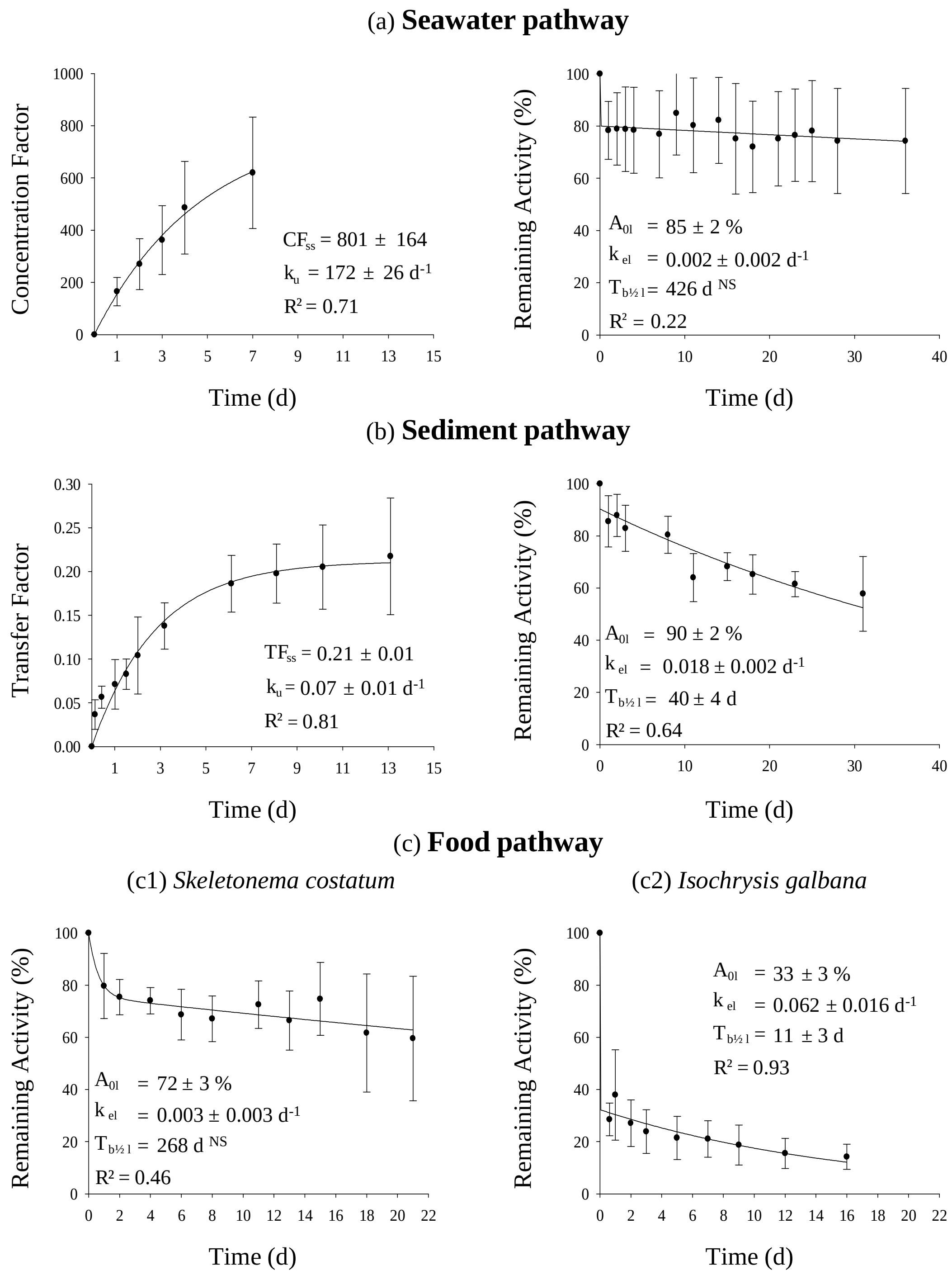

(a) Seawater pathway
Concentration Factor
CFss = 801 ± 164
ku = 172 ± 26 d-1
R² = 0.71
Time (d)
Remaining Activity (%)
A0l = 85 ± 2 %
k el = 0.002 ± 0.002 d-1
Tb½ l = 426 d NS
R² = 0.22
Time (d)
(b) Sediment pathway
Transfer Factor
TFss = 0.21 ± 0.01
ku = 0.07 ± 0.01 d-1
R² = 0.81
Time (d)
Remaining Activity (%)
A0l = 90 ± 2 %
k el = 0.018 ± 0.002 d-1
Tb½ l = 40 ± 4 d
R² = 0.64
Time (d)
(c) Food pathway
(c1) Skeletonema costatum
Remaining Activity (%)
A0l = 72 ± 3 %
k el = 0.003 ± 0.003 d-1
Tb½ l = 268 d NS
R² = 0.46
Time (d)
(c2) Isochrysis galbana
Remaining Activity (%)
A0l = 33 ± 3 %
k el = 0.062 ± 0.016 d-1
Tb½ l = 11 ± 3 d
R² = 0.93
Time (d)

Fig. 1

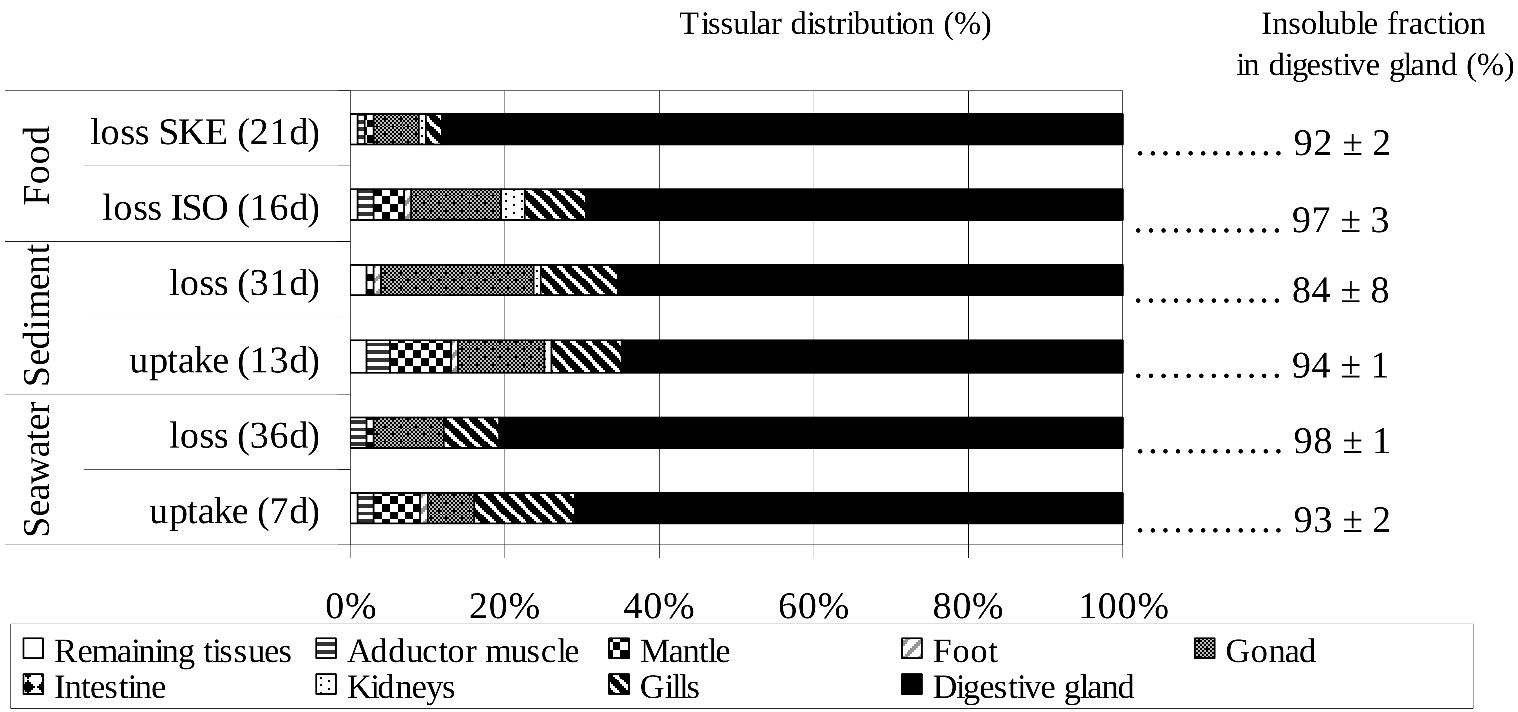
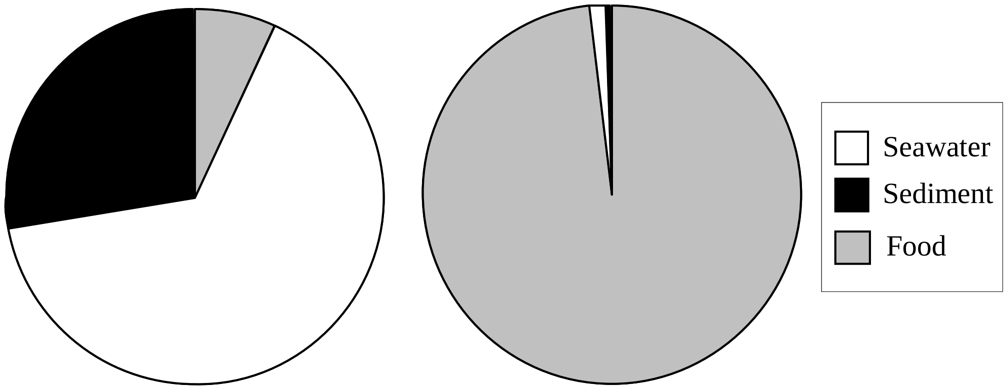

Tissular distribution (%)
Insoluble fraction in digestive gland (%)
Food
loss SKE (21d)
loss ISO (16d)
Sediment
loss (31d)
uptake (13d)
Seawater
loss (36d)
uptake (7d)
92 ± 2
97 ± 3
84 ± 8
94 ± 1
98 ± 1
93 ± 2
0%
20%
40%
60%
80%
100%
Remaining tissues
Adductor muscle
Mantle
Foot
Gonad
Intestine
Kidneys
Gills
Digestive gland

Fig. 2



(a) *I. galbana* (b) *S. costatum*

Fig. 3